Bacteriocin production as a mechanism for the antiinfective activity of *Lactobacillus salivarius* UCC118

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Edited by Todd R. Klaenhammer, North Carolina State University, Raleigh, NC, and approved March 1, 2007 (received for review January 17, 2007)

The mechanisms by which probiotic strains enhance the health of the host remain largely uncharacterized. Here we demonstrate that *Lactobacillus salivarius* UCC118, a recently sequenced and genetically tractable probiotic strain of human origin, produces a bacteriocin *in vivo* that can significantly protect mice against infection with the invasive foodborne pathogen *Listeria monocytogenes*. A stable mutant of *Lb. salivarius* UCC118 that is unable to produce the Abp118 bacteriocin also failed to protect mice against infection with two strains of *L. monocytogenes*, EGDe and LO28, confirming that bacteriocin production is the primary mediator of protection against this organism. Furthermore, *Lb. salivarius* UCC118 did not offer any protection when mice were infected with a strain of *L. monocytogenes* expressing the cognate Abp118 immunity protein AbpIM, confirming that the antimicrobial effect is a result of direct antagonism between *Lb. salivarius* and the pathogen, mediated by the bacteriocin Abp118.

infection | *Listeria* | probiotic

The gastrointestinal microbiota presents a significant barrier that must be overcome for a pathogen to initiate an infection. The concept of preventing or ameliorating intestinal infections through dietary interventions designed to manipulate commensal bacteria, or as a means of introducing transiently colonizing probiotic strains, has received much attention in recent years. Such strategies could potentially decrease antibiotic use and associated problems of antimicrobial resistance. Probiotic organisms (live bacteria that have a beneficial effect on the host when consumed in adequate amounts) have been proposed to play roles in improving digestive function, in the reduction of chronic inflammation, and in hastening recovery from intestinal disease (1–3). Studies using rodent models of infection have demonstrated a role for probiotics in the amelioration of infections caused by *Helicobacter pylori*, *Citrobacter rodentium*, and *Salmonella typhimurium*, and human trials have confirmed that the consumption of probiotic cultures can play a role in the eradication of *H. pylori* in infected patients (4–6). *In vitro* studies have indicated that the regulation of mucus production by probiotics can prevent colonization by enteropathogenic *Escherichia coli*, and there is an apparent correlation between probiotic-derived immunomodulation and the elimination of foodborne pathogens (7, 8). Although the health benefits associated with use of probiotic bacteria are well documented, their mechanistic basis remains largely unclear, and *in vivo* identification of the precise mechanistic basis of these beneficial effects remains a significant goal.

Bacteriocins are a heterogeneous family of small, heat-stable peptides with potent antimicrobial activity that are produced by many bacterial species, including many probiotic strains (9). Bacteriocins produced by Gram-positive bacteria have a bactericidal or bacteriostatic effect on other species and genera, but activity is usually limited to other Gram-positives (10). Bacteriocins have been used by the food industry to reduce the use of chemical preservatives in foods with limited shelf life, or those foodstuffs that present a high risk for pathogen contamination (11).

Lactobacillus salivarius UCC118 is a genetically well characterized strain that produces a potent broad-spectrum class II bacteriocin, Abp118, which is active against *Listeria monocytogenes* (12, 13). Abp118 is regulated by a quorum-sensing mechanism, with bacteriocin production peaking in early stationary phase cultures in response to the accumulation of an induction peptide, AbpIP. In this study we determined that *Lb. salivarius* UCC118 offers protection against *L. monocytogenes* infection in mice. Generation of a nonproducing mutant of *Lb. salivarius*, suggested that Abp118 is the basis of the antilisterial effect in this model system. This was confirmed by constructing an Abp118-immune strain of *L. monocytogenes*, which was able to overcome the protective effect conferred by UCC118.

Results

Probiotic Administration Can Enhance Resistance to Infection by *L. monocytogenes* in Mice. The number of bacteria in the murine liver and spleen 3 days after oral inoculation with *L. monocytogenes* is a well established metric of infection. When A/J mice were orally infected with *L. monocytogenes* EGDe at 2×10^9 CFU per mouse, numbers of $\approx 10^5$ reaching the liver and the spleen by day 3 is typical of a normal infection. When mice were fed a strain of *Lactococcus lactis* (a food fermentation organism not normally associated with the gastrointestinal tract, used as a control in this instance) or one of six probiotic strains (all strains administered at 1×10^9 CFU per mouse per day for 3 days) and subsequently infected with *L. monocytogenes*, *Lb. salivarius* UCC118 significantly reduced the numbers of *Listeria* in both liver and spleen on day 3 after infection (Fig. 1). *Bifidobacterium longum* JCM7050 also provided some protection in both organs, albeit not to the same level as that provided by UCC118. *Bifidobacterium breve* UCC2003 and *Bifidobacterium infantis* CCUG36569 afforded statistically significant protection against listerial splenic infection, but no significant reduction in the numbers infecting the liver was observed.

***Lb. salivarius* UCC118 Reduces *Listeria* Infection in Mice.** Recently, a luciferase-based reporter system was developed to track systemic *Listeria* infection in the murine model (14). We used a derivative of this system, in which the *hly* promoter was translationally fused

Author contributions: S.C.C., Y.L., C.U.R., P.W.O., C.H., and C.G.M.G. designed research; S.C.C. and Y.L. performed research; S.C.C., Y.L., C.U.R., P.W.O., C.H., and C.G.M.G. analyzed data; and S.C.C., Y.L., P.W.O., C.H., and C.G.M.G. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at www.pnas.org/cgi/content/full/0700440104/DC1.

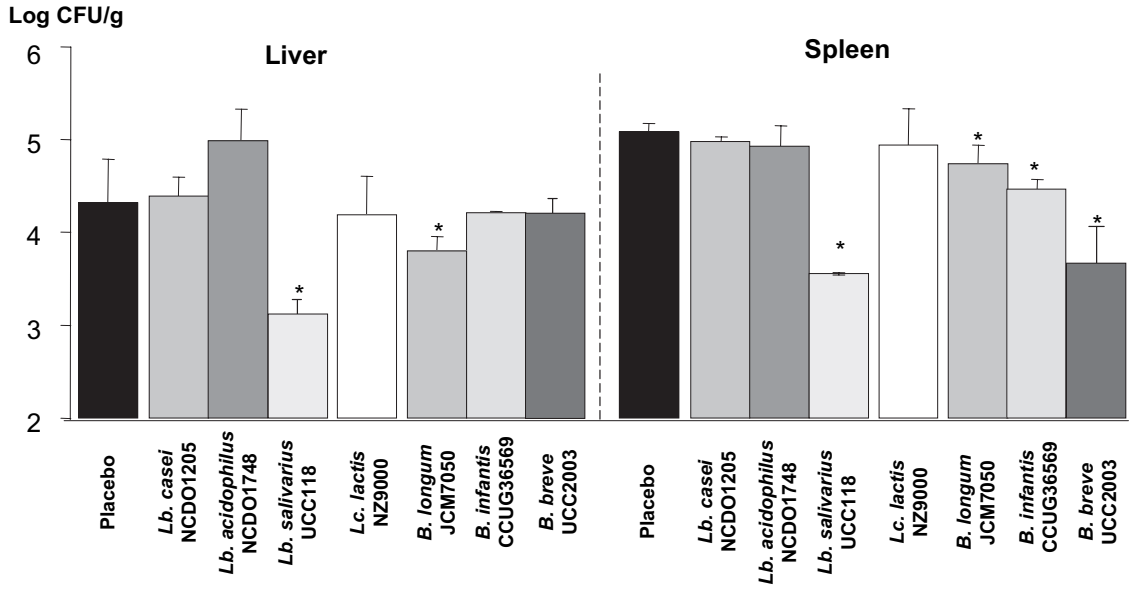

Fig. 1. *In vivo* protection against *L. monocytogenes* EGDe infection in livers and spleens after oral dosing of A/J mice with lactobacilli, *Lactococcus*, or bifidobacteria for 3 days before listerial infection. *, $P < 0.05$, indicating the statistically significant difference between the numbers of bacteria infecting organs compared with placebo-fed control mice ($n = 4$).

to *luxABCDE* and integrated into the chromosome of *L. monocytogenes* EGDe. Luciferase expression could then be used to follow organ-specific colonization of murine livers and monitor any reduction in bacterial numbers after probiotic dosing (Fig. 2*a*). Feeding mice with *Lb. salivarius* UCC118 for 6 days before infection with luminescent *L. monocytogenes* EGDe elicited a significant reduction in the amount of light emitted from livers and spleens (5.91 ± 0.82 Log_{10} relative light units per organ for placebo vs. 3.96 ± 1.18 Log_{10} relative light units per organ for the probiotic group; $P < 0.01$) (Fig. 2*a*). We also homogenized the organs and enumerated bacterial numbers, which confirm significant differences between UCC118-treated and control groups (Fig. 2*b*).

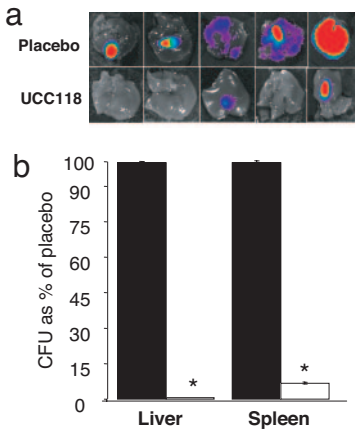

Fig. 2. Probiotic dosing of A/J mice with UCC118 reduces the level of subsequent *L. monocytogenes* infection. (*a*) Whole livers dissected from A/J mice fed probiotic *Lb. salivarius* UCC118 for 6 days before oral infection with luminescent *L. monocytogenes* EGDe:pPL2*lux*-PhlyA. Livers of UCC118-fed animals display minimal amounts of luminescence compared with those of placebo-fed mice. (*b*) Livers (from *a*) and spleens of probiotic-fed mice (open bars) are significantly less infected than organs of placebo-fed mice (filled bars). *, $P < 0.01$ compared with placebo ($n = 5$).

Influence of Dosing Regime on Protective Effect. To determine the importance of timing the dose of UCC118, mice were fed with placebo (no bacterium) or UCC118 in a number of feeding regimes (UCC118 only on the day of infection, for 1 or 2 days before and including the day of infection, and also for 2 days before, but not on the day of infection). In all cases, the numbers of bacteria in liver and spleen were determined on day 3 after infection (Fig. 3). In addition, mice were dosed with UCC118 after the infection to compare the effect of prefeeding and active consumption on the outcome of the infection (Fig. 3). All feeding regimes conferred significant levels of protection, although the mice fed on the day of infection only were protected to a lesser extent than all other treatments.

Construction of a Bacteriocin-Negative Mutant of UCC118. The ability of UCC118 to produce an antilisterial bacteriocin provides the

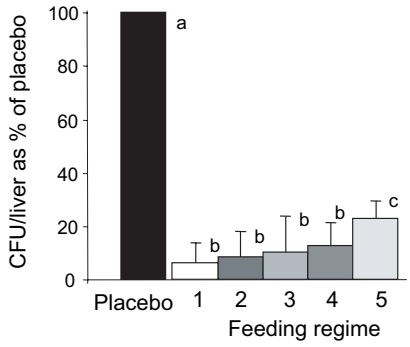

Fig. 3. Analysis of dosing regime. *Lb. salivarius* UCC118 was given orally to A/J mice for 3 days including day of infection (lane 1); 3 days before and 3 days after infection (lane 2); on the day of infection and 3 days after infection (lane 3); 2 days before infection (lane 4); and on the day of infection only (lane 5). Because of the large number of animals involved, each feeding trial was conducted with a placebo control, and results are expressed as the percentage of infection. Identical lowercase letters a–c denote no significant difference, and different letters denote a statistically significant difference ($P < 0.05$; $n = 5$).

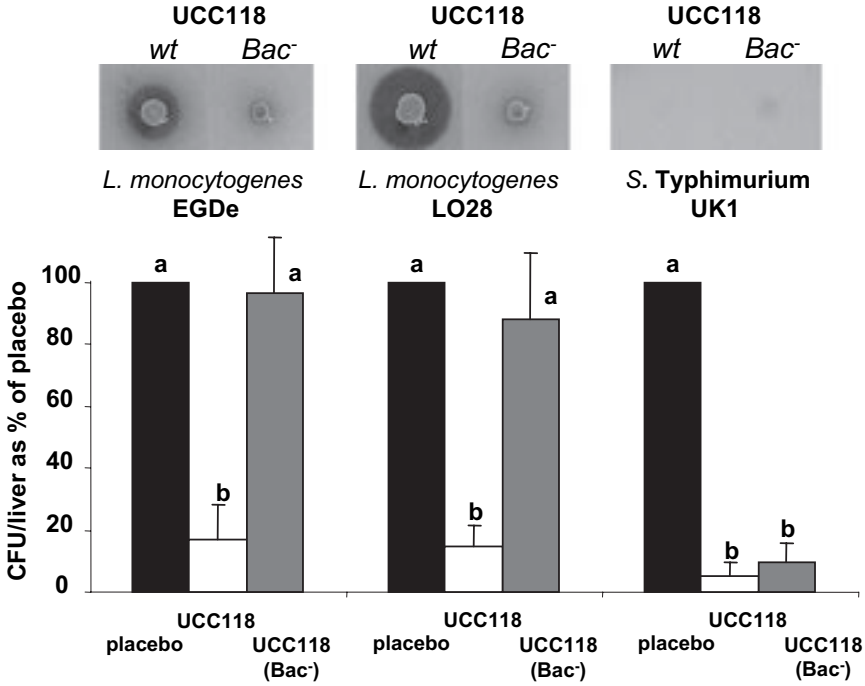

Fig. 4. Bacteriocin production mediates *Lb. salivarius* UCC118 protection against *L. monocytogenes* infection of A/J mice. A/J mice fed PBS placebo (filled bars), *Lb. salivarius* UCC118 (open bars), or UCC118Δ*abp118* (Bac$^-$) (gray bars) for 3 days before infection with *L. monocytogenes* EGDe, *L. monocytogenes* LO28, or *S. typhimurium* UK1. Because of the large number of animals involved, each feeding trial was conducted with a placebo control, and results are expressed as the percentage of infection. Identical lowercase letters a and b denote no significant difference, and different letters denote a statistically significant difference ($P < 0.001$; $n = 5$).

most obvious explanation for the observed protective effect. To eliminate bacteriocin production, we targeted the gene encoding the AbpT transporter [supporting information (SI) Fig. 6] using a plasmid integration strategy previously used successfully to inactivate multiple *Lb. salivarius* candidate adhesins (15). The genomic arrangement of UCC118Δ*abp118* was confirmed by Southern hybridization, which indicated that multiple copies of the pLS101 integration plasmid were integrated into the *abpT* locus. To determine the stability of the integration event, UCC118Δ*abp118* was grown in the absence of erythromycin for 50 generations. A combination of PCR assays and bacteriocin production assays indicated that the stability of the pLS101 integration was 96.6% over 50 generations. To examine whether the multiple-copy integration of pLS101 into the *abpT* locus resulted in any obvious change of phenotype, we examined the competitive growth dynamics by inoculating an equal number of cells of strain UCC118 and UCC118Δ*abp118* into MRS free of antibiotics. The ratio of UCC118 to UCC118Δ*abp118* did not change significantly over a 50-h growth period, suggesting that the fitness of the mutant relative to the parent was unaltered.

Bacteriocin Production Mediates Protection Against *Listeria* Infection. *L. monocytogenes* EGDe and *L. monocytogenes* LO28 are sensitive to the Abp118 bacteriocin, as determined by well diffusion assay (Fig. 4). Mice were administered either *Lb. salivarius* UCC118 or UCC118Δ*abp118* for 3 days before oral infection with both strains of *Listeria*. UCC118Δ*abp118* did not confer protection against either strain of *L. monocytogenes* (Fig. 4). Infection levels in mice fed UCC118 were again significantly reduced compared with placebo controls, whereas mice fed the bacteriocin-negative UCC118Δ*abp118* displayed levels of infection similar to placebo-fed mice (Fig. 4). This trial was repeated by using *S. typhimurium* UK1, which is insensitive to the Abp118 bacteriocin (Fig. 4). We observed that although *Lb. salivarius* UCC118 can confer protection against *S. typhimurium* UK1, this effect was not linked to bacteriocin production, because the bacteriocin-negative strain also conferred similar levels of protection. This indicated that, in the case of *S. typhimurium* UK1, protection is not a consequence of direct antagonism between probiotic and pathogen (as expected, because Abp118 does not inhibit salmonellae). This implies that the ability to produce a bacteriocin is not central to all antiinfection mechanisms and that the protective effects are likely to be more complex than simple antimicrobial production and may be pathogen-specific.

Confirmation of Direct Antagonism as the UCC118 Anti-*Listeria* Mechanism. Although the most obvious explanation for the observed protection is the direct killing of *Listeria* by UCC118, the bacteriocin could also be involved in mediating transient colonization or could be acting as an antigen or signaling molecule to the murine immune system. To eliminate these other possibilities, we created a strain of *L. monocytogenes* EGDe immune to Abp118 by heterologous expression of the associated immunity determinant, *abpIM*, a strategy previously shown to confer immunity on heterologous strains (16). The Abp118-immune phenotype was confirmed by using an agar-diffusion assay (Fig. 5). The immunity conferred is bacteriocin-specific, will protect against only direct antagonism by UCC118, and would not protect against induced host or commensal flora defenses. Mice received UCC118 for 3 days before oral infection with *L. monocytogenes* EGDe(*abpIM*). We observed that the UCC118 protective effect previously observed against EGDe (and reconfirmed in this experiment; data not shown) was lost when EGDe(*abpIM*) was used as the infectious strain. This confirms that the role of bacteriocin in mediating UCC118 protection against *Listeria in vivo* is based on direct antagonism between probiotic and pathogen.

Discussion

Bacterial infections, and in particular intestinal infectious diseases, are a major cause of morbidity and mortality. In the

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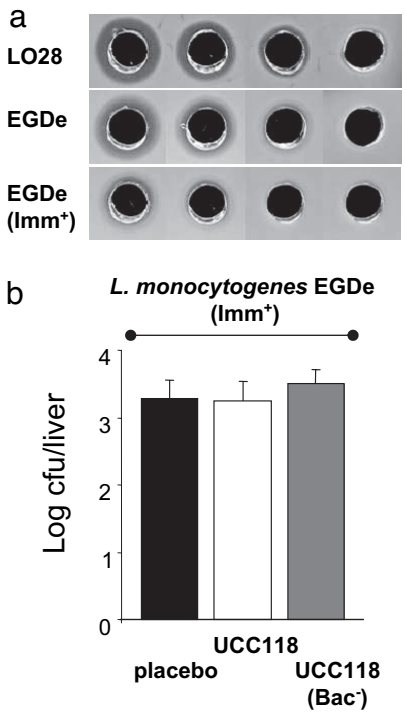

Fig. 5. Immunity to Abp118 overcomes the protective effect of UCC118. (*a*) Confirmation of immune phenotype of *L. monocytogenes* EGDe(*abpIM*) by using an agar-diffusion assay. *L. monocytogenes* LO28 and *L. monocytogenes* EGDe are susceptible to the Abp118 bacteriocin. (*b*) A/J mice fed PBS placebo, *Lb. salivarius* UCC118, or UCC118Δ*abp118* for 3 days before infection with *L. monocytogenes* EGDe(*abpIM*).

United States there are ≈76 million cases of foodborne illness every year, with 5,000 deaths (17), and, of those deaths resulting from bacterial pathogens, ≈40% are due to listeriosis caused by *L. monocytogenes.* Although the availability of antibiotics to treat these infections has significantly improved health and well-being, this has not been without limitations. Overuse of antibiotics, with an associated development and dissemination of specific genetic resistance mechanisms, has contributed to the emergence of antibiotic-resistant bacteria. As a result, alternative prophylactic and therapeutic strategies are urgently required.

One of the potential alternatives to using antibiotics either prophylactically or therapeutically to prevent infection is the exploitation of bacteriocins, bacterially derived peptide antimicrobials. Despite considerable promise exhibited against foodborne pathogens both *in vitro* and in food systems, *in situ* bacteriocin production by a probiotic strain has never been shown to confer a protective effect against infection with a gastrointestinal pathogen (18). However, it has been established that production of the bacteriocin mutacin is important for long-term colonization of the mouth by a *Streptococcus mutans* strain that has been genetically modified to be deficient in lactate dehydrogenase activity (19). This strain has significantly reduced cariogenic potential and can act to replace the existing *S. mutans* populations in the oral cavity in long-term colonization studies (termed replacement therapy). The example in the current article is significantly different in that it involves only transient colonization and represents direct antagonism between a probiotic and pathogen, rather than intraspecies competitive exclusion. Although a number of mechanisms have been postulated to explain probiotic effects, many of these have been investigated solely *in vitro* or (in the case of immunomodulatory effects) involve building hypotheses based on data from numerous diverse experiments. In the present study a single mechanism has been demonstrated to be solely responsible for a defined protective and antimicrobial effect.

There are several lines of evidence to support the importance of bacteriocin production in the antilisterial effect. First, the other strains tested were incapable of preventing *Listeria* infection in mice to the same extent, with most strains demonstrating no efficacy in this model. Of the probiotic strains tested, only *Lb. salivarius* UCC118 secreted a factor with direct antilisterial activity. The work is in agreement with recent studies that suggest that particular probiotic strains differ greatly in their *in vivo* effects (8). In the current study, although systemic infection was not completely prevented after probiotic treatment, a consistent reduction of between one and two logs in the organs was achieved, depending on individual experiments. It must be borne in mind that individual mice received a standard oral dose of 2×10^9 *L. monocytogenes* cells to achieve consistent infection but that these doses are far in excess of that likely to be ingested by humans through contaminated food sources. We would expect a much more significant protective effect with a lower infectious dose of *Listeria*, but the limited sensitivity of the murine model does not allow us to test this theory.

A second line of evidence that supports direct antagonism as a mode of action involved examination of various dosing regimes, in which we determined that mice that received a dose of *Lb. salivarius* only 30 min before listerial infection are nonetheless significantly protected. This suggests that protection is achieved within a very short timescale, ruling out a mechanism involving a specific induction of the host immune system.

Third, a stable mutant of *Lb. salivarius* UCC118 that fails to produce the bacteriocin Abp118 also failed to protect mice against *L. monocytogenes* infection, further supporting the theory that bacteriocin production is the primary mediator of protection against this organism. It is possible that the bacteriocin could operate by interacting with the established host flora. If that were the case, one would not expect the protective effect to depend on the relative sensitivity of the infectious organism to the bacteriocin. However, when we expressed the Abp118 immunity gene in *L. monocytogenes* EGDe we observed that the resulting strain is equally infectious, regardless of whether the infected mice have been dosed with *Lb. salivarius* UCC118 before infection. This supports the proposal that the bacteriocin acts directly against the target cell and not through an intermediate mechanism.

Fourth, we also demonstrate that this mechanism is specific for target organisms that demonstrate bacteriocin sensitivity, because the protection observed against a naturally bacteriocin-resistant foodborne pathogen, *S. typhimurium* UK1, was clearly independent of bacteriocin production and may involve a different mechanism of competitive exclusion, or perhaps may indicate an immunomodulatory mechanism.

The use of bacteriocin-producing cultures may be a particularly useful approach for a rare but deadly disease such as listeriosis, where prophylactic chemotherapy or vaccination is not an option, e.g., in pregnant women. Furthermore, the identification of bacteriocin-mediated probiotic protection could aid the development of alternative bacteriocin-based therapies. It is interesting in this regard that a recent study by Mota-Meira *et al.* (20) demonstrated that mice infected i.p. with *Staphylococcus aureus* and subsequently injected with mutacin, a bacteriocin produced by *S. mutans*, are protected from subsequent bacterial challenge. We have also demonstrated that the topical use of a purified bacteriocin, lacticin 3147, can act to prevent bovine mastitis after deliberate infection (21). It will be important in regard to using bacteriocin-producing strains as therapeutic agents to establish whether the transient introduction of a bacteriocin-producing organism has consequences for the normal commensal flora. Despite a growing acceptance of the evidence from numerous trials that probiotics can play a

positive role in human physiology, the precise mechanistic basis of probiotic action remains a major research goal (22). Demonstration of a key antimicrobial and protective mechanism *in vivo* will contribute to validating the growing clinical and commercial relevance of probiotic bacteria in human health. The work reported here suggests that to develop probiotic bacteria with clinical potential as antiinfective agents, *in vitro* selection protocols based on key properties, such as bacteriocin production, will increase the likelihood of *in vivo* efficacy.

Materials and Methods

Bacterial Strains, Media, and Growth Conditions. *L. monocytogenes* EGDe and *L. monocytogenes* LO28 were grown in Brain Heart Infusion broth (Oxoid, Basingstoke, England) (supplemented with 7.5 μg/ml chloramphenicol where required) and shaken aerobically at 37°C. *S. typhimurium* UK1 was grown in Tryptone Soy broth (Merck, Darmstadt, Germany) and shaken aerobically at 37°C. *Lb. salivarius* subsp. *salivarius* UCC118 strains were grown in MRS broth (Oxoid) (supplemented with 5 μg/ml erythromycin where required) anaerobically at 37°C for 36 h and then subcultured for a further 24 h.

Animal Studies. Harlan A/J mice were used for *in vivo* studies. Mice were housed under pathogen-free conditions in a dedicated facility. Mice were fed with food and water unless otherwise stated. At least five mice were used per bacterial strain. On the day of infection, mice received 2×10^9 CFU of *L. monocytogenes* EGDe by oral pipette. Three days after infection, mice were killed by cervical dislocation, livers and spleens were excised, and CFU per organ was determined. All animal procedures were performed according to national ethical guidelines.

Probiotic Dosing of Mice to Assess the Effect on Listerial Infection *in Vivo*. Animal trials were performed to determine whether prefeeding mice a strain of *Lactobacillus* or bifidobacteria conferred protection against subsequent infection with *L. monocytogenes*. Harlan A/J mice (aged 7 weeks) were administered lactobacilli or bifidobacteria (1×10^9 CFU per mouse) by oral pipette for 3 or 6 days before oral infection with *L. monocytogenes* EGDe (2×10^9 CFU per mouse). A placebo group of mice were fed PBS (Gibco, Paisley, Scotland) for the 3 days before infection. Bioluminescence levels in livers were imaged by using a Xenogen IVIS 100 system (Xenogen, Alameda, CA) (Fig. 2).

Determination of the Importance of *Lb. salivarius* UCC118 Dosing Regime. To determine the importance of timing the probiotic dose, a range of dosing regimes was tested (Fig. 3). Mice were orally dosed with 1×10^9 CFU of *Lb. salivarius* UCC118 for 3 days before listerial infection, 2 days before infection but not on the day of infection, 3 days before infection and during the listerial infection period, on the day of infection and during the listerial infection period, or on the day of listerial infection only. When *Lb. salivarius* UCC118 was administered on the day of infection, this was performed 30 min before mice were orally infected with *L. monocytogenes*.

Creation of Bacteriocin-Negative *Lb. salivarius* UCC118 by Plasmid Integration at the *abpT* Gene. Plasmid integration was conducted as reported previously, with minor modifications (15) (see *SI Text*).

Assessment of the Role of *Lb. salivarius* UCC118 Bacteriocin Production in Protection Against *Listeria* Infection. Mice were orally dosed with 1×10^9 CFU of *Lb. salivarius* UCC118 or *Lb. salivarius* UCC118Δ*abp118* for 3 days before listerial infection (Fig. 4). Control mice received a PBS placebo for 3 days before listerial infection.

Creation of *L. monocytogenes* EGDeabpIM. To confirm whether the ABP118 bacteriocin mediated protection against *L. monocytogenes* infection, the immunity protein ApbIM was expressed in *L. monocytogenes* EGDe to create a bacteriocin-resistant strain *L. monocytogenes* EGDe*abpIM*. The procedure was carried out by using an adapted protocol devised by Horton *et al.* (23).

Statistical Analysis. Numerical results are expressed as Log CFU per organ or as a percentage of CFU per organ infecting placebo. Error bars in the figures represent standard deviations. Student's *t* test was performed to determine statistical significance.

We thank J.-P. van Pijkeren for his advice and assistance in creating the Bac$^-$ strain of *Lb. salivarius* UCC118. This research was supported by the Irish Government under the National Development Plan (2000–2006) and by Science Foundation Ireland through a Centre for Science, Engineering, and Technology award to the Alimentary Pharmabiotic Centre.

1. Savaiano DA, AbouElAnouar A, Smith DE, Levitt MD (1984) *Am J Clin Nutr* 40:1219–1223.
2. Di Giacinto C, Marinaro M, Sanchez M, Strober W, Boirivant M (2005) *J Immunol* 174:3237–3246.
3. Gotteland M, Brunser O, Cruchet S (2006) *Aliment Pharmacol Ther* 23:1077–1086.
4. Johnson-Henry KC, Nadjafi M, Avitzur Y, Mitchell DJ, Ngan BY, Galindo-Mata E, Jones NL, Sherman PM (2005) *J Infect Dis* 191:2106–2117.
5. Silva AM, Barbosa FH, Duarte R, Vieira LQ, Arantes RM, Nicoli JR (2004) *J Appl Microbiol* 97:29–37.
6. Mack DR, Michail S, Wel S, McDougall L, Hollingsworth MA (1999) *Am J Physiol* 276:G941–G950.
7. Jijon H, Backer J, Diaz H, Yeung H, Theil D, McKaigney C, DeSimone C, Madsen K (2004) *Gastroenterology* 126:1358–1373.
8. O'Hara AM, O'Regan P, Fanning A, O'Mahony C, MacSharry J, Lyons A, Bienenstock J, O'Mahony L, Shanahan F (2006) *Immunology* 118:202–215.
9. Cotter PD, Hill C, Ross RP (2005) *Nat Rev Microbiol* 3:777–788.
10. Klaenhammer TR (1993) *FEMS Microbiol Rev* 12:39–85.
11. Abee T, Krockel L, Hill C (1995) *Int J Food Microbiol* 28:169–185.
12. Claesson MJ, Li Y, Leahy S, Canchaya C, Van Pijkeren JP, Cerdeno-Tarraga AM, Parkhill J, Flynn S, O'Suliivan GC, Collins JK, *et al.* (2006) *Proc Natl Acad Sci USA* 103:6718–6723.
13. Dunne C, O'Mahony L, Murphy L, Thornton G, Morrissey D, O'Halloran S, Feeney M, Flynn S, Fitzgerald G, Daly C, *et al.* (2001) *Am J Clin Nutr* 73:386S–392S.
14. Bron PA, Monk IR, Corr SC, Hill C, Gahan CGM (2006) *Appl Env Microbiol* 72:2876–2884.
15. Van Pijkeren JP, Canchaya C, Ryan KA, Li Y, Claesson MJ, Sheil B, Steidler L, O'Mahony L, Fitzgerald GF, Van Sinderen D, O'Toole PW (2006) *Appl Environ Microbiol* 72:4143–4153.
16. Flynn S, Van Sinderen D, Thornton GM, Holo H, Nes IF, Collins JK (2002) *Microbiology* 148:973–984.
17. Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, Griffin PM, Tauxe RV (1999) *Emerg Infect Dis* 5:607–625.
18. Kirkup BC (2006) *Curr Med Chem* 13:3335–3350.
19. Smith L, Orugunty RS, Hillman JD (2006) *Research and Applications in Bacteriocins*, eds Riley MA, Gillor O (Horizon Bioscience, Norfolk, UK).
20. Mota-Meira M, Morency H, Lavoie MC (2005) *J Antimicrob Chemother* 56:869–871.
21. Ryan MP, Meaney W, Flynn J, Ross RP, Hill C (1998) *Appl Environ Microbiol* 64:2287–2290.
22. Marco ML, Pavan S, Kleerebezem M (2006) *Curr Opin Biotechnol* 17:204–210.
23. Horton RM, Cai Z, Ho SN, Pease LR (1990) *Biotechniques* 8:528–534.